functional homotetrameric channels that can be activated in a cooperative manner by cAMP or cGMP binding to the cyclic nucleotide-binding domains (CNBD) included in each subunit. Our aim was to kinetically further dissect the molecular mechanism leading to channel activation upon ligand binding and to channel deactivation upon ligand removal.
CNGA2 channels, expressed in Xenopus oocytes, were studied in excised patches by measuring simultaneously ligand binding/unbinding and activation/deactivation by means of confocal patch-clamp fluorometry under steady-state and non-steady state conditions (182 or 277 frames per second). Concentration jumps of a fluorescent cGMP derivative (Biskup et al., Nature, 446(7134): 440-3, 2007) were applied using a fast piezoelectric system. Surprisingly, the unbinding was concentration dependent while deactivation was concentration independent. The unbinding was approximately 100 times faster from fully liganded channels in comparison with the unbinding from lowly liganded channels. The obtained data were analyzed by global fits to various types of Markovian state models. The additional information of unbinding and deactivation allowed us to refine the previously determined C4L-Model (Biskup et al., Nature, 446(7134): 440-3, 2007). To account for the very fast unbinding at saturating ligand concentrations, the C4L-Model had to be expanded: When fully liganded, the channel adopts an open state which allows, upon ligand removal, a very fast unbinding of all four ligands. In contrast, from partially liganded states this fast unbinding is occluded. Our results suggest an additional pathway for rapid ligand unbinding for the fully liganded channel.

1439-Pos Board B331
Probability Fluxes and Transition Paths in a Markovian Model Describing Complex Subunit Cooperativity in HCN2 Channels
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Hyperpolarization-activated cyclic nucleotide-modulated (HCN) ion channels are voltage-gated tetrameric cation channels that generate pacemaker activity in neurons and cardiomyocytes. Activation of these channels can be enhanced by the binding of adenosine-3',5'-cyclic monophosphate (cAMP) to an intracellular cyclic-nucleotide binding domain in each of the four subunits. Based on previously determined rate constants for a complex Markovian model describing the gating of homotetrameric HCN2 channels (Kusch et al., Nat. Chem. Biol. 8, 162-9, 2012), we analyzed probability fluxes within this model, including unidirectional probability fluxes. Following the rules of the transition path theory, we analyzed the transition paths in our model for channel activation, following a jump to a defined ligand concentration from zero, and for channel deactivation, following a jump from the ligand concentration back to zero. Three ligand concentrations were considered. The time-dependent probability fluxes quantify the contributions of all 13 transitions of the model to channel activation. The binding of the first, third and fourth ligand evoked robust channel opening whereas the binding of the second ligand obstructed channel opening similar to the empty channel. Our analysis of the net probability fluxes revealed pronounced hysteresis for channel activation and deactivation. These results provide insight into the complex cooperative interaction of the four subunits equal by sequence, leading to pronounced differences in the subunit function.

1440-Pos Board B332
A Canine CNGB3 Channelopathy Suggests that Changes in Calcium Homeostasis Result in Progressive Loss of Cone Function
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Canine day-blindness, a model for human achromatopsia, is associated with loss of cone function due either to the deletion or a missense Asp (D) 262 to Asn (N) mutation in CNGB3. Asp 262 resides in an acidic motif in the S2 transmembrane helix conserved in all CNG channel subunits and members of the Shaker K+ superfamily. Tetrameric cyclic nucleotide-gated (CNG) channels are formed from CNGA3 and CNGB3 subunits and transduce light information in cone photoreceptor outer segments. In canine day-blindness, the CNGB3-D262N mutation leads to loss of cone function between 4 and ~10 weeks suggesting progressive physiological changes. We investigated the missense mutation using the human CNGB3, previously used in gene therapy to restore cone function in young dogs (Hum Mol Genet 2010 19: 2581). Canine CNGA3 was co-expressed with hCNGB3; the most significant functional difference between homomeric and heteromeric currents was an ~10 fold increase in PCa/PNa in heteromeric channels. Co-expression of cCNGA3 with hCNGB3-D262N (canine numbering) result in the absence of functional heteromeric channels with evidence of some homomeric CNGA3 channels. We suggest that alterations in calcium homeostasis associated with the missense mutation in CNGB3 contribute to the loss of cone function. We generated mutations in the Asp residues in CNGA3 channels. We investigated substitutions in the three Asp residues in S2 and all mutations examined resulted in the loss of channel function underscoring the essential role for these residues in channel function. Studies in voltage-gated channels show electrostatic interactions between the acidic residues in S2 and residues in S3 and S4 transmembrane domains. Our future experiments will explore the role of these acidic residues in intra-subunit helical interactions using mutagenesis and molecular modeling.

1441-Pos Board B333
Monitoring CNGA3 and CNGB3 Subunit Expression in Retinas of Day-Blind Canines with Inherited Deletion or Missense CNGB3 Asp262Asn Mutations Show Progressive Loss of Both CNGB3 and CNGA3 Expression
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Cone cyclic nucleotide-gated (CNG) channels are heteromeric, composed of CNGA3 and CNGB3 subunits. Inherited canine day blindness is similar to human achromatopsia and results in loss of cone function. Affected dogs have inherited deletion (-/-) or missense (m/m) Asp262Asn mutations in the CNGB3 gene. Cone ERG studies from m/m dogs show early, progressive loss of cone function with complete loss by ~6-weeks. Immunohistochemical analysis of m/m and -/- retinal tissues using an anti-CNGA3 antibody show outer segment expression until ~6 weeks; no immunoreactivity is observed at 1 year. A polyclonal antibody was generated against the C-terminus of canine CNGB3 to investigate expression in the m/m dogs. Immunohistochemistry on 6-week, 8-week and one-year old m/m retinas showed no expression of CNGB3. Immunoblots of retinal homogenates from m/m and -/- mutant dogs showed no reactivity although the antibody recognizes the mutant protein as demonstrated with heterologously-expressed human B3-D262N (canine numbering). Previous qRT-PCR studies examined expression of both CNGA3 and CNGB3 mRNA. Levels of CNGA3 were similar for unaffected, m/m and -/- retinas; CNGB3 mRNA levels were similar for unaffected and m/m dogs but, as expected, levels in -/- retinas were non-detectable. The affected m/m and -/- dogs provide models for inherited human channelopathies including achromatopsia with the potential for direct retinal examination of affected dogs during development and following gene therapy. Results with m/m dogs show that the CNGB3 subunits are degraded; importantly, both the m/m and -/- dogs loose expression of CNGA3 in outer segments. This surprising result implies that an intact CNGB3 is a requirement for CNGA3 expression, a result not predicted or replicated in heterologous expression of CNGA3 channels.

Cardiac, Smooth, & Skeletal Muscle Electrophysiology I

1442-Pos Board B334
Endogenous VIP May Contribute to Vagally Induced Electrophysiological Changes in Canine Atria
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Background: There has been increasing evidence that complex interactions among the various components of intracardiac neural network play an important role in atrial fibrillation (AF). Perfusion of vasoactive intestine polypeptide (VIP), a neural polypeptide, co-released with acetylcholine from intrinsic cardiac neurons during vagal stimulation, was shown to shorten the action potential duration (APD), decrease the intraatrial conduction velocity (CV), and promote induction of AF. However, the effect of endogenous VIP remains unclear. Methods: In 6 isolated arterially perfused canine left atria, high-resolution optical mapping techniques with di-4-ANEPPS and blebbistatin were used to measure APD and CV during fat-pad ganglion plexus stimulation (GPS, 30Hz, 10.2 ± 2.3Volt validated with blockage of atrioventricular conduction), at during H9935, a VIP antagonist (1 μM) perfusion. The atria were paced at 200beats per minute. Metoprolol (1.8μM) was used to block the sympathetic effects. Results: Average APD was shorter (21%) during GPS compared to the baseline (100 ± 8ms vs. 126 ± 7ms, $p<0.05$), and average CV was slower than baseline (87 ± 10cm/sec vs. 103 ± 13cm/sec, $p<0.05$), which recovered within 2 min (APD: 128 ± 8ms, $p<0.05$; CV: 105 ± 13cm/sec, $p<0.05$). With H9335, the APD shortening effect (17%) of GPS persisted (GPS, 105 ± 14ms, vs. 127 ± 8ms at baseline and 125 ± 10ms after recovery, $p<0.05$) with a trend towards being less pronounced as compared to GPS effect without H9335